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6. AUTHOR(S) RANDOLPH V. LEWIS				
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13. ABSTRACT (Maximum 200 words)  The aciniform glands from <i>Argiope trifasciata</i> were used to construct a cDNA library. The library was probed with various DNA probes based on known spider silk protein sequences. A large 9kb clone was identified and partial sequencing has been done. The C-terminal sequence is clearly related to the same sequence in a number of other spider silks. The 5' end of the clone still appears to be repetitive sequence and thus it is unlikely to be a full-length clone. We have completed about 4.5 kb of sequence at this point. The remaining sequence is being completed. There were an additional 6 clones of smaller length found but all contain the same sequence as the largest one. The sequence to date shows some homology to other spider silks but is distinctly different. It appears to have a repeat sequence of A cDNA library from <i>Nephila clavipes</i> has been constructed from the aciniform/piriform glands and is in the process of screening. This will be used to compare the aciniform silk sequences as well as to identify the piriform silk sequences.				
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Report period: 6/1/01-11/30/02

Title: Sequence of Spider Aciniform and Piriform Silks

Grant Number: DAAD19-01-1-0569

Institution: University of Wyoming

Author of Report: Randolph V. Lewis

Manuscripts:

To be submitted:

(2003) Gatesy, J., Hayashi, C.Y., and Lewis, R.V. Codon Usage in Spider Silk Proteins from Diverse Species (to be submitted),

(2003) Gatesy, J., Hayashi, C.Y., and Lewis, R.V. Sequence of Spider Aciniform Silk Protein

Submitted:

(2002) Lewis, R.V. Sequences, Structures and Properties of Spider Silks in Elastic Proteins: Structure, Properties and Biological Roles, Cambridge University Press, Cambridge, UK

(2002) Lewis, R.V. Silk Fibroin Proteins: Structure, Function and Evolution, Biopolymers, ed. by Alexander Steinbüchel, Wiley-VCH Verlag GmbH

(2003) Hayashi, C.Y., Gatesy, J., and Lewis, R.V. Convergence of Spider Silk Protein Sequences with Other Proteins (submitted to Science)

Published:

(2002) Spider Silk: Lessons from Nature's Loom, Shane R. Nelson<sup>1</sup> and Randolph V. Lewis Agro Food Industry Hi Tech 13: 33-38.

Thesis:

None

Scientific Personnel and Degrees Awarded during this period:

Dagmara Motriuk, PhD student

Shane Nelson, PhD student

Congzhou Liu, M.S. awarded

Justin Jones, Research Scientist

Inventions:

Spider Silk Protein Encoding Nucleic Acids, Polypeptides, Antibodies and Methods of Use Thereof-  
Application submitted

Specific Aims:

The goal was to clone and sequence genes for aciniform and piriform silks from orb weavers. These silks have not been studied to any large extent and based on their amino acid composition may contain new motifs.

Results:

We have made significant progress toward achieving the first goal of this project. A cDNA library was constructed from mRNA of the aciniform glands of locally caught *Argiope trifasciata*, as these glands can be removed from the other glands relatively easily in this species. The library was probed with radioactive DNA probes based on the known sequences of the other silks we have cloned and sequenced.

A number of positive clones were identified with the largest being about 9 kb. This clone as well as the rest of greater than 3 kb were subjected to initial DNA sequencing from both ends of the insert.

These data indicated that all of the clones appeared to be of the same protein as the sequences were identical at the C-terminal end (3') and had the same repeat sequence internally. So efforts were directed toward the largest clone containing the most information. To date we have obtained nearly 4.5 kb of data. However, it has been very difficult to sequence as the transposon mediated method we use does not seem to insert in much of the sequence. We are currently working to overcome this problem. Very recently, with another silk sequence we have determined that, for unknown reasons, those clones with the transposon in the insert grow much slower than those without. Thus we have to let the colonies grow for over 36 h to see these colonies as opposed to the usual 16 h timeframe.

The sequence we have obtained shows an apparent repeat of 200 amino acids, nearly the longest of any silk protein yet known. It shows motifs seen in other silks but is predominantly a new repeat sequence. The motifs seen in other silks are the GGX motif, the proline containing motifs, generally GPGXX, and the  $\beta$ -sheet motif, which is serine rich as opposed to most silks where these are alanine rich. There are also a higher percentage of hydrophilic and charged residues than seen in other orb-weaver silks. In this regard it appears more like the major ampullate silk from a primitive spider than any other silk.

We have also constructed a cDNA library from *Nephila clavipes* that includes both the aciniform and piriform glands as they cannot be separated due to their being intertwined. This library is being probed with the same probes as were used for the *Argiope* library. We will construct probes based on the current aciniform sequence as probes as well. We expect to complete the current aciniform silk in the next few months and have partial sequences for other silks in the same time frame.